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Short communication

Antitumor and anti-inflammatory activities of polysaccharides isolated from *Ganoderma lucidum*

SONIAMOL JOSEPH[1]
BABY SABULAL[1,*]
VARUGHESE GEORGE[1,3]
KUTTIKKADAN RONY ANTONY[2]
KAINOOR KRISHNANKUTTY JANARDHANAN[2]

[1] *Phytochemistry and Phytopharmacology Division, Tropical Botanic Garden and Research Institute, Palode Thiruvananthapuram-695562, India*

[2] *Amala Cancer Research Centre Amala Nagar, Thrissur-680555, India*

[3] *Amity Institute for Herbal and Biotech Products Development, Peroorkada Thiruvananthapuram-695005, India*

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In this study, polysaccharides were isolated from *Ganoderma lucidum* (*Polyporaceae*) and their antitumor and anti-inflammatory activities were investigated using *in vivo* models. Potential antitumor activity was shown by *G. lucidum* polysaccharides (GLP) against solid tumor induced by Ehrlich's ascites carcinoma cells. GLP at 100 mg kg^{-1} body mass showed 80.8 and 77.6 % reduction in tumour volume and tumour mass, respectively, when administered 24 h after tumour implantation. Again, GLP at the same dose but when administered prior to tumour inoculation, showed 79.5 and 81.2 % inhibition of tumour volume and tumour mass, respectively. GLP showed significant dose-dependent activity in carrageenean-induced (acute) and formalin-induced (chronic) inflammation assays. At 100 mg kg^{-1}, GLP exhibited 57.6 and 58.2 % inhibition in carrageenean-induced and formalin-induced assays, respectively.

Keywords: *Ganoderma lucidum* (*Polyporaceae*), polysaccharides, anti-inflammatory activity, antitumor activity

Fungi are an attractive source of physiologically functional foods and drug precursors, displaying a wide range of pharmacological activities such as anticancer, anti-inflammatory, antitumor and immunomodulating effects (1). Antitumor polysaccharides such as krestin, lentinan and schizophyllan have been isolated from various fungi. Polysaccharides from natural sources have diverse immunomodulatory activities both *in vitro* and *in vivo*. They are known modulators of cellular and humoral immunity. Polysaccharides derived from fungi are known for their antitumor and immunomodulating properties. They exert antitumor activity through activation of various immune responses in the host. Further, different strains of *Basidiomycetes* produce polysaccharides of varying properties (2, 3).

Ganoderma lucidum, a fungus grown on rotten wood, is a well known source of therapeutic agents. *G. lucidum*, commonly known as 'Reishi' or 'Lingzhi', has been extensively used in oriental folk medicine for thousands of years to treat various diseases, in-

* Correspondence; e-mail: sabulal@gmail.com

cluding cancer (4). Further, it has been reported to have antioxidant, anti-inflammatory, antitumor, antihypertensive, antihistaminic and antihepatotoxic effects. Cytotoxic triterpenoids and immunomodulating polysaccharides are the major chemical constituents of *G. lucidum* (5). *In vitro* studies showed that the administration of polysaccharides isolated from *G. lucidum* spores could effectively inhibit growth in mice with transplanted S180 sarcoma (6). Here, we report the *in vivo* anti-inflammatory and antitumour activities of the polysaccharide isolates (GLP) of *G. lucidum* from southern India.

EXPERIMENTAL

Plant material

G. lucidum (*Polyporaceae*) fruiting bodies were collected from Thrissur district in Kerala, India. A voucher specimen was deposited at the Herbarium of the Centre for Advanced Studies in Botany, University of Madras, Chennai, India.

Isolation of G. lucidum *polysaccharides*

Fruiting bodies of *G. lucidum* (200 g) were cut into small pieces, dried at 40–50 °C for 48 h and powdered. Powdered mushroom was defatted with petroleum ether. Defatted *G. lucidum* powder was extracted with doubly distilled water (1:25, *m*/*V*) at 80 °C for 8 h. This extract was filtered and concentrated at 40 °C in a rotary vacuum evaporator (Büchi Rotavapor R-114, Switzerland). Ethanol (95 %) was added to the concentrated aqueous extract (5:1, *V*/*V*), stirred vigorously and kept at 4 °C for 48 h. The polysaccharides which precipitated out were collected by centrifugation at 16,800 × *g* for 20 min. The precipitate obtained was washed with ethanol and dried. The residue thus obtained was dissolved in deionised water, loaded on a DEAE cellulose column and eluted with deionised water. The fractions obtained were treated with diluted HCl followed by anthrone reagent. Green colour indicated the presence of polysaccharides. All anthrone positive fractions were combined and re-peatedly shaken with Sevag's reagent to remove the proteins (7). After protein removal, the polysaccharides were again re-precipitated with chilled ethanol (95 %), and this precipitate was dissolved in deionised water and dialyzed (1:1.00,000) in water for 48 h. On testing with phenol/H_2SO_4 reagent, this fraction showed a violet colour indicating polysaccharides. It was then evaporated at low temperature and lyophilized to obtain *G. lucidum* polysaccharide fraction (GLP, 900 mg, 0.4 %) as a light brown powder.

Animals

Male Swiss albino mice were procured from the Small Animal Breeding Centre, Veterinary College, Thrissur, India. Animals were kept for a week under environmentally controlled conditions before experimentation. They were fed a standard diet and water *ad libitum*. Mice weighing 25 ± 2 g were used. All animal experiments were carried out with the approval and according to the guidelines of the Institutional Animal Ethics Committee.

Cell line

Ehrlich's ascites carcinoma (EAC) cell line was obtained from Adyar Cancer Institute, Chennai, India. The cells were maintained by intraperitoneal inoculation of 1×10^6 viable cells in mice.

Determination of antitumor activity

Antitumor activity of GLP was determined using two solid tumor models.

Antitumor activity of GLP when administered after tumor cell implantation. – Animals were divided into five groups of six animals each (8). Viable EAC cells 1×10^6 in 0.1 mL PBS were transplanted subcutaneously into the right groin of mice. GLP (25, 50 and 100 mg kg^{-1} body mass) was administered 24 h after tumor cell implantation and continued for ten consecutive days. The control group received normal saline instead of GLP. Cyclophosphamide (25 mg kg^{-1}) was used as the reference standard drug. GLP and standard drug were administered using a 22-guage oral cannula for mice. Tumor development in animals of each group was determined by measuring the diameter of tumor growth in two perpendicular planes using vernier calipers twice a week for five weeks. Tumor volume was calculated using the formula $4/3\pi r_1^2 r_2$, where r_1 is the minor diameter and r_2 is the major diameter. At the end of the fifth week, animals were sacrificed under anesthesia using diethyl ether. The tumors were extirpated, weighed and the percent inhibition was calculated (9).

Antitumor activity of GLP when administered prior to tumor cell implantation. – GLP (25, 50 and 100 mg kg^{-1} body mass) was administered for ten consecutive days followed by tumor cell implantation which was carried out 1 h after the last dose of GLP administration cells (8). Animals were kept untreated after tumor implantation. Control group received only normal saline. Cyclophosphamide (25 mg kg^{-1}) was used as the reference drug. Initiation of tumor growth was observed. Tumor diameter was measured using vernier calipers twice a week for a period of three weeks. At the end of the third week, the animals were sacrificed, tumors were extirpated, weighed and percentage inhibition was calculated (9).

Determination of anti-inflammatory activity

Carrageenean-induced edema. – Animals were divided into four groups of six animals each. Acute inflammation was produced in all animals by subplantar injection of 20 μL freshly prepared 1 % suspension of carrageenean in normal saline to the right hind paw of mice (10). Paw thickness was measured using vernier calipers before and after carrageenean injection in each group. Animals were premedicated with GLP (25, 50 and 100 mg kg^{-1} body mass), and with the reference drug diclofenac (10 mg kg^{-1}), orally 1 h before carrageenean injection.

Formalin-induced edema. – Animals were treated exactly as in the carrageenean-induced method but instead of carrageenean, freshly prepared 2 % formalin in normal saline (20 μL) was used as the edematogenic agent. Drug treatment was continued for six consecutive days (11). In both carrageenean-induced and formalin-induced models, the

degree of edema formation was determined as the increase in paw thickness. The percent inhibition was calculated as reported by Ajith and Janardhanan (12).

Statistical analysis

The data were statistically analyzed using Student's *t*-test. All data were represented as mean ± SD.

RESULTS AND DISCUSSION

Polysaccharides isolated from fruiting bodies of *G. lucidum* (GLP) showed significant antitumour activity against solid tumor. GLP, when administered 24 h after tumor implantation at doses of 25, 50 and 100 mg kg^{-1} body mass, inhibited 64.3, 73.4 and 80.8 % of tumor volume and 58.3, 67.6 and 77.6 % of tumor mass, respectively, comparable to 71.8 and 73.8 % achieved by 25 mg kg^{-1} cyclophosphamide, resp. (Table I). The tumor volume and mass in GLP treated groups of animals were significantly lower than in the control group. At 100 mg kg^{-1}, GLP inhibited tumor proliferation as effectively as the standard reference drug cyclophosphamide at 25 mg kg^{-1}. Pretreatment with GLP was

Table I. Antitumor activity of GLP against solid tumor induced by Ehrlich's ascites carcinoma cells

Treatment	Initial tumor volume (cm^3)[a]	Initial tumor mass (g)[a]
Antitumor activity of GLP when administered after tumor implantation		
Control[b]	0.2790 ± 0.0191	4.19 ± 0.55
Cyclophosphamide (25 mg kg^{-1})	0.0786 ± 0.0173[c]	1.10 ± 0.21[e]
Polysaccharide		
25 mg kg^{-1}	0.0996 ± 0.0230[d]	1.75 ± 0.27
50 mg kg^{-1}	0.0741 ± 0.0134[c]	1.36 ± 0.28
100 mg kg^{-1}	0.0535 ± 0.0029[e]	0.93 ± 0.07[e]
Antitumor activity of GLP when administered prior to tumor implantation		
Control[b]	0.2037 ± 0.0280	4.74 ± 0.29
Cyclophosphamide (25 mg kg^{-1})	0.3600 ± 0.0080[e]	0.98 ± 0.02[e]
Polysaccharide		
25 mg kg^{-1}	0.0891 ± 0.0084[e]	2.0 ± 0.24[e]
50 mg kg^{-1}	0.0640 ± 0.0085[e]	1.6 ± 0.30[e]
100 mg kg^{-1}	0.0416 ± 0.0113[e]	0.89 ± 0.02[e]

[a] Mean ± SD (n = 6).
[b] Normal saline, 0.8 mL per animal.
Significant difference with respect to control: [c] $p < 0.01$, [d] $p < 0.005$, [e] $p < 0.001$.

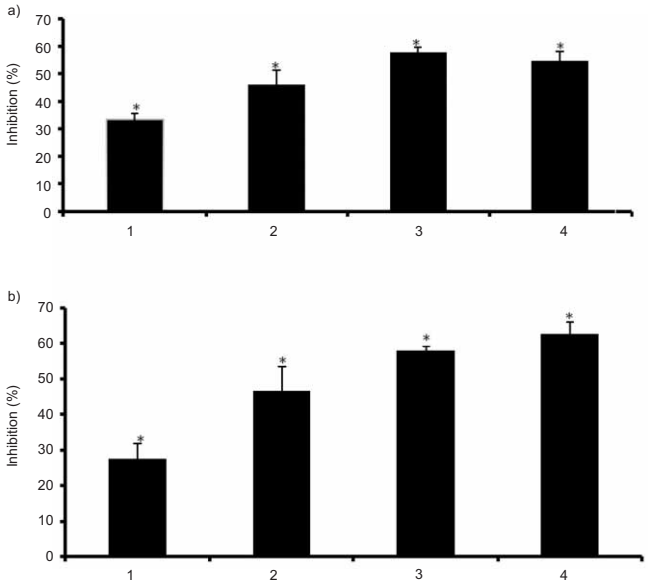

Fig. 1. Effect of GLP on: a) carrageenean-induced acute inflammation, b) formalin-induced chronic inflammation; *G. lucidum* polysaccharide fractions 1: 25; 2: 50; 3: 75 mg kg^{-1}; 4: diclofenac (10 mg kg^{-1}). SD bars, $n = 6$. Significant difference with respect to control: * $p < 0.01$.

also effective in inhibiting tumor proliferation induced by EAC cells. Administration of the same doses of GLP for ten consecutive days prior to tumor inoculation inhibited 56.3, 68.4 and 79.5 % of tumor volume and 56.8, 65.2 and 81.2 % of tumor mass, respectively, whereas the standard drug cyclophosphamide (25 mg kg^{-1} body mass) inhibited 82.3 % tumor volume and 79.2 % tumor mass (Table I).

At 25, 50 and 100 mg kg^{-1} body mass, GLP inhibited acute inflammation induced by carrageenean and chronic inflammation induced by formalin in a dose dependent manner (Figs. 1a,b). At 100 mg kg^{-1} body mass GLP showed activity comparable to that of diclofenac (10 mg kg^{-1}) in both assays. Thus, the present study has confirmed strong anti-inflammatory activity of the polysaccharides isolated from the southern Indian accession of *G. lucidum*.

The main components of *G. lucidum* polysaccharides are β-1,3 and β-1,6-D-glucans (Fig. 2). β-D-glucan is a carbohydrate polymer with chains of glucose molecules linked together by β-glycosidic linkages (13). *G. lucidum* is traditionally used for the prevention and treatment of a large number of diseases, including many forms of cancer. *G. lucidum* extracts and polysaccharides were also proven to have, along with antitumor, also immune potentiating activities (14, 15). *G. lucidum* polysaccharides were shown to induce apoptosis, inhibit cell proliferation and suppress cell migration of highly invasive human prostate cancer cells. The mechanism of antitumor activity of *G. lucidum* polysaccharides proceeded through stimulation of host defence responses (14, 15). Our earlier

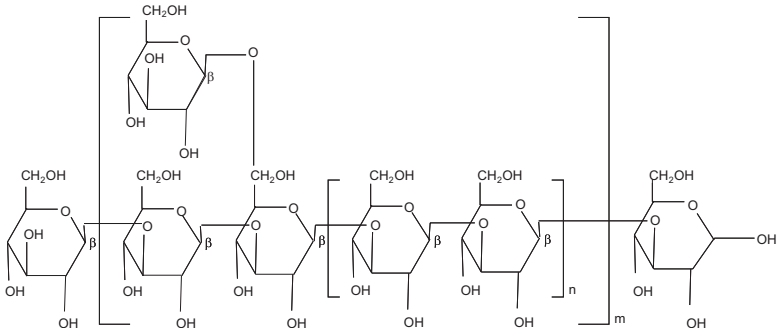

Fig. 2. Repeating unit of *Ganoderma lucidum* polysaccharides.

studies reported antitumor, antioxidant (16), anti-inflammatory and antinociceptive activities (17) exhibited by crude extracts of southern Indian accessions of *G. lucidum*.

CONCLUSIONS

The present study has shown that an isolated polysaccharide fraction of *G. lucidum* has marked antitumor and anti-inflammatory activities. These results on polysaccharides and previous data on extracts suggest the therapeutic potential of southern Indian strains of *G. lucidum*.

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SAŽETAK

Citostatsko i protuupalno djelovanje polisaharida biljke *Ganoderma lucidum*

SONIAMOL JOSEPH, BABY SABULAL, VARUGHESE GEORGE, KUTTIKKADAN RONY ANTONY i KAINOOR KRISHNANKUTTY JANARDHANAN

U radu je ispitano *in vivo* citostatsko i protuupalno djelovanje polisaharida (GLP) izoliranih iz biljke *Ganoderma lucidum* (*Polyporaceae*). Ispitivani polisaharidi pokazali su potencijalno antitumorsko djelovanje na Ehrlichov ascitesni tumor. GLP su u dozi od 100 mg kg^{-1} tjelesne mase inhibirali volumen tumora za 80,8, a njegovu masu za 77,6 %, kada su primijenjeni 24 h nakon implantacije tumora. Ako se GLP daju u istoj dozi prije inokulacije tumora, inhibiraju volumen tumora za 79,5, a njegovu masu za 81,2 %. GLP pokazuju značajno, o dozi ovisno, protuupalno djelovanje u karagenan testu (akutna

upala) i formalin testu (kronična upala). U dozi od 100 mg kg^{-1}, GLP inhibiraju upalne procese za 57,6 odnosno 58,2 % u testu s karagenanom, odnosno formalinom.

Ključne riječi: *Ganoderma lucidum* (*Polyporaceae*), polisaharidi, protuupalno djelovanje, citostatsko djelovanje

Phytochemistry and Phytopharmacology Division, Tropical Botanic Garden and Research Institute Palode, Thiruvananthapuram-695562, India

Amala Cancer Research Centre, Amala Nagar, Thrissur-680555, India

Amity Institute for Herbal and Biotech Products Development, Peroorkada, Thiruvananthapuram-695005 India